Inhaled corticosteroids in children: use and effects of early treatment on asthma and lung function

Prevalence of asthma and the impact of severity in early life on later asthma in childhood

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CONTENTS

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2 LIST OF PAPERS

The present thesis is based on the four papers listed below.

Paper I

Asthma in every fifth child in Oslo, Norway: a 10-year follow up of a birth cohort study.
Karin C. Lødrup Carlsen, Geir Håland, Chandra Sekhar Devulapalli, Monica Cheng Munthe-Kaas, Morten Pettersen, Berit Granum, Martinus Løvik and Kai-Håkon Carlsen. Allergy 2006 Apr;61(4):454-60

Paper II

Effect of inhaled steroids on lung function in young children: a cohort study.
Chandra Sekhar Devulapalli, Geir Håland, Morten Pettersen, Kai-Håkon Carlsen, and Karin C. Lødrup Carlsen. Eur Respir J. 2004 Jun;23(6):869-75.

Paper III

Severity of obstructive airways disease by age 2 years predicts asthma at 10 years of age.
Chandra Sekhar Devulapalli, Karin C. Lødrup Carlsen, Geir Håland, Monica Cheng Munthe-Kaas, Morten Pettersen, Petter Mowinckel and Kai-Håkon Carlsen. Thorax 2008; 63(1):8-13. Epub 2007 Jul 5.

Paper IV

No evidence that early use of inhaled corticosteroids reduces current asthma at 10 years of age.
Chandra Sekhar Devulapalli, Karin C. Lødrup Carlsen, Geir Håland, Monica Cheng Munthe-Kaas, Morten Pettersen, Petter Mowinckel and Kai-Håkon Carlsen. Respir Med. 2007 Aug; 101(8):1625-1632. Epub 2007 May 21.

3 ABBREVIATIONS

BHR	bronchial hyper responsiveness
BO	bronchial obstruction
CI	confidence interval
ECA	Environment and childhood asthma
FEV_1	forced expiratory volume in one second
FEF_{50}	forced expiratory flow at 50 % of vital capacity
FVC	forced vital capacity
GA^2LEN	Global Allergy and Asthma European Network
GINA	Global Initiative for Asthma
ICS	inhaled corticosteroids
Ig	immunoglobulin
ISAAC	International Study of Asthma and Allergies in Children
NPV	negative predictive value
PPV	positive predictive value
OAD	obstructive airways disease
OR	odds ratio
aOR	adjusted odds ratio
PD_{20}	provocation dose of methacholine causing 20% fall in FEV_1
RAST	radio allergo sorbent test
rBO	recurrent bronchial obstruction
RCT	randomized clinical trial
ROC	receiver operated characteristic
s-ECP	serum eosinophilic cationic protein
SPT	skin prick test
TFV	tidal flow volume
t_{PTEF}/t_E	ratio of time to peak expiratory flow to total expiratory time

4 SUMMARY

Treatment for a chronic disorder may be regarded as a part of the environmental influences, but is not commonly looked upon as such. Although inhaled corticosteroids (ICS) for the last decades have been the treatment of choice for mild persistent to severe persistent asthma in accordance with the guidelines, little is known especially from long term studies regarding possible modifying effects of early ICS treatment on development of asthma in children. Understanding how treatment influences development of later asthma has important implications for prevention of the disease.

After several decades with an increase in prevalence of asthma in the Western world, some recent reports suggest that prevalence of childhood asthma has reached a plateau in some European countries. Some studies, on the other hand, have found a steady rise in the prevalence rates.

Although there is evidence to show that severe asthma in childhood tends to persist in adulthood, it is not well known whether severity of obstructive airways disease (OAD) in early life has impact on later asthma, the understanding of which may improve follow-up of children with high risk for persistent asthma.

Aims of the thesis:

The main objective of the present thesis was to assess if early treatment with inhaled corticosteroids could modify disease progression in childhood.

1. To investigate the prevalence of recurrent bronchial obstruction (rBO) and asthma in children in a general urban population.
2. To determine how often inhaled corticosteroids were used for treatment of obstructive airways disease in childhood.
3. To assess if ICS treatment had an effect on lung function in young children with recurrent bronchial obstruction (rBO).

4. To define a severity score for severity of obstructive airways disease during the first two years of life and assess if the severity score can be used as a tool to predict asthma in school children.
5. To explore if early ICS treatment in children with obstructive airways disease during the first two years of age can modify occurrence of current asthma in school children.

Study design

The present study is part of a 10 year follow-up of children in the prospective birth cohort, the Environment and Childhood Asthma (ECA) study in Oslo. Briefly, a cohort of 3,754 children was established at birth in Oslo in 1992. The study design had three main phases. The initial phase was to obtain background information including family history of diseases, environment, and clinical characteristics of the newborn baby as well as lung function measurements shortly after birth in 803 children. The second phase covered the first 2 years of life which included questionnaires, and a nested case-control study at 2 years including children with physician confirmed recurrent (>1) bronchial obstruction (rBO) or persistent bronchial obstruction (>4 weeks) (n=306) and age-matched controls (n=306). The third phase was the 10 year follow-up study attended by 1019 of 1215 children (84 %) with lung function measurements at birth and/or a clinical two-year investigation.

Subjects and methods

For aim 1 (to investigate the prevalence of recurrent bronchial obstruction by two years of age) and aim 2, all children from the entire cohort (3697 of 3754 with complete questionnaire data by two years of age) who had completed all five follow-up questionnaires as well as all children defined with rBO who had attended at least one visit were assessed. To assess the prevalence of asthma by 10 years of age, the 616 of 803 subjects who had lung function measurements performed shortly after birth were reinvestigated at the age of 10 years with detailed clinical examinations including parental interview, lung function measurements and

skin prick test (paper I). At birth, they corresponded to the entire birth cohort of 3754 children. History of asthma, asthma during the last 12 months, and allergic skin sensitization at 10 years of age were assessed.

For aim 3, 54 children with rBO (with and without ICS treatment) and 15 controls with tidal flow volume measurements upon presentation of disease (mean age 11 months) and two years of age were studied (paper II).

For the aims four and five, 459 subjects (239 and 220 with and without rBO, respectively at two years of age) from the nested case control study who attended 10 year follow-up and underwent clinical examination, parental interview, treadmill test and bronchial hyper responsiveness test to methacholine were studied. A severity score at two years of age was calculated by frequency, persistence of symptoms of bronchial obstruction and hospital admissions due to OAD and was used to predict asthma at 10 years of age (paper III). The same study population was studied to assess the risk of current asthma at 10 years of age in children who received ICS compared to those who did not by two years of age (paper IV).

Results

Recurrent bronchial obstruction or asthma in children

Of all healthy children enrolled at birth in the study (3697 of 3754 with complete questionnaire data by two years of age), 306 subjects had documented symptoms of recurrent bronchial obstruction by two years of age, corresponding to prevalence of 8.3 % with rBO in young children.

In 10-year-old children, the lifetime prevalence of asthma was 20.2 %, current asthma 11.1 %, doctors' diagnosis (as reported by the parents) of asthma 16.1 %, wheeze ever 30.3 % and allergic skin sensitization 29.3 % at 10 years of age. Our finding of 20.2 % lifetime prevalence of asthma among 10 year old children represented the highest number ever

reported in Scandinavia by year 2005. However, the increase in asthma prevalence was not accompanied by parallel increase in allergic skin sensitization.

Use of inhaled corticosteroids in young children

From the entire cohort (3697 of 3754 with complete questionnaire data by two years of age), 77 children corresponding to a prevalence of 2.1 % and 64 (21 %) of children with rBO had received ICS treatment by two years of age.

Effect of inhaled steroids on lung function in young children

Baseline lung function assessed by tidal breathing (the ratio of time to peak expiratory flow to total expiratory time (t_{PTEF}/t_E)) at the debut visit was significantly lower in children who later received ICS as compared to those who did not, but there were no significant differences between the treatment groups in baseline lung function at the two year visit. The mean difference in baseline t_{PTEF}/t_E (change from first to second visit) was significantly higher (borderline) in the ICS-treated group only and correlated significantly with duration of ICS treatment.

Impact of severity in early life on later asthma

Severity score at two years of age was significantly higher among rBO children who developed current asthma at 10 years of age compared to rBO children without current asthma (5.5, 4.9-6.1 *versus* 4.0, 3.6-4.5, respectively, $p<0.001$). Receiver operated characteristic (ROC) analysis, positive and negative predictive values demonstrated the applicability and value of the severity score with optimal cut off value at six. Furthermore, children with severity score above five had more often severe bronchial hyperresponsiveness (BHR) than children with a lower or 0 score (22.2 % versus 8.5 %, respectively, $p=0.0041$). The risk (odds ratio, 95 % confidence intervals) of current asthma among rBO subjects when compared to no BO subjects was 7.9, 4.1-15.3 whereas the risk of current asthma among rBO

subjects with severity score above five was 20.2, 9.9-41.3 compared to no BO children.

Impact of early ICS treatment on the prognosis of asthma

ICS treatment in the first two years of life did not reduce the risk of current asthma eight years later in our observational birth cohort study. Propensity modeling was used to compensate for non-randomness and adjust for severity of disease at two years of age. In rBO children logistic regression analyses identified male gender and severity score at two years as significant risk factors for current asthma at 10 years of age, whereas the use of ICS treatment before two years of age was not significantly (borderline) associated with current asthma at 10 years of age.

Conclusions

1. Recurrent bronchial obstruction was seen in 8.3 % children in the present prospective birth cohort by two years of age.

   Furthermore, every fifth 10 year old child in the city of Oslo at some time had asthma.
2. The use of ICS (as reported by parents) corresponded to 2.1 % of all children in the cohort study and 21 % of children with rBO by two years of age.
3. Lung function appeared to improve in children using ICS from the start of symptoms of OAD until two years of age, mostly in children with the longest duration of treatment.
4. A scoring system based on severity and frequency of obstructive airways disease during the first two years of life predicted current asthma at 10 years of age.
5. Use of ICS during the first two years of life in children with obstructive airways disease did not reduce asthma present eight years later.

5 REVIEW OF THE LITERATURE

5.1 History of asthma

The actual term asthma is a Greek word that is derived from the verb *ααζειν* (aazein), meaning to exhale with open mouth, to pant (1). The expression asthma appeared for the first time in Homer's Iliad (1), with the meaning of a short-drawn breath, but the earliest text where the word is found as a medical term is the Corpus Hippocraticum in 450 BC (1). Hippocrates thought that the spasms associated with asthma were more likely to occur in tailors, anglers, and metalworkers. Six centuries later, Galen wrote much about asthma, noting that it was caused by partial or complete bronchial obstruction. In 1190 AD, Moses Maimonides, an influential medieval rabbi, philosopher, and physician, wrote a treatise on asthma, describing its prevention, diagnosis, and treatment (2). In the 17th century, Bernardino Ramazzini noted a connection between asthma and organic dust. Its status as a modern disease, however, dates to the autopsy series of the Paris hospitals in the 1810s, where bronchial asthma, cardiac asthma, and other forms of shortness of breath were redefined as pathological entities. Asthma was treated with a variety of available remedies from morphine to tobacco in the nineteenth century to steroids and beta-agonist inhalers in the twentieth. The use of bronchodilators started in 1901, but it was not until the 1960s that the inflammatory component of asthma was recognized, and anti-inflammatory medications were added to the management.

5.2 Asthma description

Asthma is a chronic disorder defined by its clinical, physiological and pathological characteristics, but since the aetiology and pathogenesis is not entirely known, much of its definition is descriptive. Based on the functional consequences of airway inflammation, an operational description of asthma according to Global initiative for asthma (GINA) (3) is:

"Asthma is a chronic inflammatory disorder of the airways in which many cells and cellular elements play a role. The chronic inflammation is associated with airway hyper responsiveness that leads to recurrent episodes of wheezing, breathlessness, chest tightness, and coughing particularly at night or in the early morning. These episodes are usually associated with widespread, but variable, airflow obstruction within the lung that is often reversible either spontaneously or with treatment." Asthma is characterised by relapsing nature of symptoms occurring either spontaneously or in response to external stimuli. The disease has a varying natural history, ranging from a transient disease of childhood through to persistent severe disease throughout life.

5.3 Asthma in children

Asthma is the most common chronic disease among children, and the most frequent cause of hospital admissions in childhood (4;5). The disease has great bearings on the individual suffering from the disease, their family as well as for the society at large. The large increase in asthma seen worldwide over the last decades has recently been characterized as an epidemic (6), with causes largely unknown. In a recent publication from International Study of Asthma and Allergies in Childhood (ISAAC) Phase-III conducted worldwide, Asher and co-workers (7) reported increasing prevalence of asthma in 6-7 year old children compared to 13-14 year age-group during the five year period (1997-2002-3). The majority of participating centres in this global study reported increased asthma prevalence in childhood (7). Pearce and co-workers (8) using ISAAC questionnaires found increases in asthma prevalence in Africa, Latin America, and parts of Asia indicating that the global burden of asthma is continuing to rise. The same study, however showed decreases in asthma prevalence in English speaking countries and Western Europe and increases in prevalence in regions where prevalence was previously low (8). Ait Khaled and co-workers (9) found that urbanized centers in African countries reported higher prevalence of asthma similar to that of

Westernized nations, with considerable variations in the prevalence rates between the reporting centers within the same country and between the countries.

Wheezing phenotypes and asthma in preschool children

The onset of asthma commonly occurs during the preschool years (10). However, the identification of children with asthma remains problematic in preschool age group (11). The clinical syndrome we recognize as asthma in school age does not develop in all infants and children with wheezing illnesses.

Diagnosis of asthma in preschool children is particularly difficult as viral induced episodic wheezing commonly occurs in children younger than three years of age. Three categories of wheezing have been described for children;

1) *Transient early wheezing* is often limited to the first three years with no symptoms later in life. Transient wheezing is often associated with prematurity and parental smoking.
2) *Persistent early-onset wheezing*: Symptoms usually begin before three years of age persisting into school age. The children have recurrent episodes of wheezing associated with acute respiratory viral infections, but no evidence of atopy or family history of atopy.
3) *Late onset wheezing/asthma*: Children have asthma that often persists throughout childhood into adult life. They usually have an atopic background, often with eczema, and airway pathology that is characteristic of asthma.

These wheezing phenotypes are retrospectively categorized partly based on lung function parameters and are used widely for epidemiological research (12). However, these asthma phenotypes are of limited help in caring for an individual patient in clinical practice. Recently, a report (13) studying characteristics of wheezing in 12-59 months old children has identified a subgroup with severe intermittent wheezing characterized by atopic features and

substantial illness-related symptom burden despite prolonged periods of wellness.

Differences between adult and childhood asthma

The pathology of asthma is often less progressed in children; with a smaller degree of airway remodelling that may be reflected in differing responses to treatment (14). Furthermore, asthma in childhood is generally less severe than in adults, with intermittent and mild persistent disease characterising the majority of patients (14). However, paediatric asthma is frequently associated with atopy (the predisposition to develop immunoglobulin-E against specific allergens) and other atopic diseases like eczema and rhinitis.

Apart from difficulties in diagnosing asthma in preschool children, major challenges in paediatric asthma include development of methods of easily assessing lung function and non-invasive methods of assessing inflammation in asthmatic children.

Asthma from childhood to adulthood

Burrows and co-workers (15) have hypothesized as early as in 1977 that paediatric respiratory illness represents an important risk factor for the development of obstructive airway diseases in adult life. It is suggested that these childhood respiratory illnesses cause the adult lung to be unusually susceptible to the adverse effects of a variety of bronchial irritants and infectious agents (15). Other studies (16;17) have confirmed these findings. Godden et al (16) found that the subjects who had asthma in childhood were more likely to wheeze in adult life. Similarly, in an unselected birth cohort from Dunedin, New Zealand, more than 25 % who had wheezing in childhood persisted in to adulthood or relapsed after remission until age 26 years (17). The factors predicting persistence or relapse were sensitization to house dust mites, airway hyperresponsiveness, female sex, smoking, and early age at onset (17). It is therefore suggested that the outcomes in adult asthma may be determined primarily in early childhood (15-17). Segala and co-workers (18) found that asthma beginning in childhood and relapsing in adulthood had greater frequency of asthma

attacks, earlier onset of allergy and more obstructed airflow than those with adult-onset asthma.

5.4 Prevalence of childhood asthma

The prevalence of asthma symptoms in children varies from one to more than 30 % in different populations and is increasing in most countries, especially among children (7). The highest prevalence has been reported in English speaking countries (Australia, New Zealand, and England) and lower levels in Scandinavian countries (19). A Norwegian cross-sectional study (20) using ISAAC questionnaires has shown prevalence of asthma to be 13.8 % in 9-11 year old children during the year 2000, slightly increased from 13.2 % during the year 1995. In the last mentioned study, increase in prevalence of asthma was observed only in boys while it reached a plateau in girls during the period 1995-2000. On the other hand, an another Norwegian study (21) showed significantly more prevalent asthma among girls compared to boys. In the latter study, asthma was reported by 8.5 % and 12.2 % among girls and 7.1 % and 7.0 % among boys in the age group 13-16 and 17-19 years, respectively. Burr et al (22) from a study of 12 year old British school children reported continued increased prevalence of asthma ever to 27.3 % and current wheeze to 19.7% during the year 2003.

Physician diagnosed asthma varies considerably from country to country, with UK study (23) reporting highest levels (around 21%) to 4-7% in a Finnish study (24) during mid-1995-1997. In the studies of Norwegian school children, physician diagnosed asthma was around 8-9% (25-28) while it was only 5.1% in Russian children bordering to Scandinavia during 1994-5 (29).

'Wheeze ever' was reported in 11% Norwegian children in Oslo (28) and 13-20 % in Finnish children (24) while it was significantly higher (49%) in British children (30). In a recent Norwegian study by Tollefsen et al (21), current wheeze was reported by 29.0 % and 33.5 % of girls and 20.4 % and 22.1 % of boys in the age group 13-16 and 17-19 years, respectively.

Recent reports from some European countries (31;32) suggested that a plateau was reached regarding rates of childhood asthma prevalence, after several decades with an increase of asthma observed in the Western world (33;34). On the other hand, some studies (35;36) have found increases in asthma prevalence, during the periods from 1986-2001 in 7-17 year old Danish children (35) and 1992-1999 in 5-14 year old German children (36).

It should be noted that all the above mentioned studies are cross-sectional surveys as there is limited knowledge available from prospective cohort studies.

5.5 Early risk factors for later asthma in childhood

The majority of children with asthma have asthma-like symptoms during the first few years of life (37;38). However, predicting asthma in school age from wheezing illness in early life is challenging since the aetiology of wheezy lower respiratory tract disease and the outcomes differ in early childhood (12;39;40).

Several studies (41-43) have found association with development of childhood asthma and parental history of asthma and/or allergic rhinoconjunctivitis. Bener et al (41) have shown that history of asthma in father, mother, siblings and asthma in second degree relatives were significant predictors for paediatric asthma. Furthermore, longitudinal studies (43;44) have shown that personal history of allergic rhinitis, atopic dermatitis and allergic sensitization especially to house dust mites, were independent risk factors for the development of childhood asthma. Kulig et al (45) from the German birth cohort, the MAS study found that persistent food sensitization in combination with a positive atopic family history was a strong predictor for the development of asthma and allergic rhinitis at five years of age. Moreover, food allergy has been implicated as a strong risk factor for later asthma life-threatening asthma in children (46).

There is now convincing evidence that children who develop lower respiratory symptoms during infection with respiratory syncytial virus (RSV) in early life are at increased risk of

developing asthma-like symptoms later in life (47-49). Furthermore, in children born into asthmatic/atopic families, viral exposures in early life were associated with asthma symptoms (50), especially symptomatic rhinovirus illnesses (51).

There is strong evidence for a causal relationship between environmental tobacco smoke exposure and asthmatic symptoms (52-54), whereas the evidence between environmental tobacco smoke exposure and development of allergy was much weaker (52). A prospective birth cohort study (55) demonstrated that the residential dampness problems increased the risk of bronchial obstruction in young children. Similarly, Pekkanen et al (56) in a population-based case control study found that moisture damage and mould growth in the main living quarters were associated with the development of asthma in early childhood.

The mechanism of BHR in early childhood and later development of asthma is not yet well known. In a study by Saga et al (57), bronchial reactivity to inhaled methacholine during the infantile period was studied using the transcutaneous partial pressure of oxygen method and children were followed 10 years for the development of asthma. They found that infants with a clinical diagnosis of bronchiolitis or wheezy bronchitis and who showed BHR in the infantile period, had increased risk of later asthma (57).

Risk factors as predictors

Although risk factors for development of childhood asthma were extensively studied, only few studies (58;59) have focused on using risk factors to predict later asthma.

From the Tucson Children's Respiratory Study, Castro-Rodriguez and co-workers (58) developed two clinical indices at three years of age to define risk of asthma in school age. Their indices included six parameters (frequency of wheezing during the first three years of life, history of eczema, parental history of asthma, eosinophilia, allergic rhinitis and wheezing without colds). They found active asthma on at least one time-point between 6-13 years in 76% and 59% of children with their positive stringent and loose index models,

respectively. Clough and co-workers (59) from a prospective longitudinal study developed models to predict 12 months persistent wheezing in infant wheezers (three to 36 months of age) with at least one atopic parent. They have included in their models parameters such as personal atopy, parental atopy, number of siblings and immune markers measured in blood. Their best model had a maximum positive predictive value of 76% and a negative predictive value of 68% in identifying a positive clinical outcome (child requiring prophylactic anti-asthma treatment after one year). In the last mentioned study, the follow-up was only after one year duration.

The knowledge of possible impact of severity of OAD in early life on later asthma is rather limited. Reijonen and colleagues from a randomized controlled follow-up study (60) demonstrated increased risk of later asthma among hospital admitted young children with wheezing. Apart from this study, none of the studies included severity of frequency and persistence and /or hospital admissions for OAD in early life in their predictive models.

5.6 Asthma and lung function in infancy through childhood

Asthma may be associated with impairment of lung function as shown in many studies. The knowledge about lung function in childhood is increasing especially from prospective, longitudinal birth cohort studies (61-63). In the Tucson Children's Respiratory Study (61;64), the chest compression technique to obtain partial maximum flow-volume curves together with assessments of the tidal breathing curve were studied in children between the ages of 1 and 6 months before any recorded lower respiratory illnesses. The authors found that the children who had an lower respiratory illness during the first year of life had significantly lower mean values for several parameters (61). Moreover, when data for the first three years of life were assessed, the association became clearer, especially for parameters obtained from forced expiratory flow-volume loops (64). Follow-up data at six years of age have shown that the children who had wheezing before three years of age but not at the age of six ("transient

wheezers") had diminished airway function (maximal expiratory flow at functional residual capacity [*V'*max FRC]) than those who did not wheeze during the first 3 years of life (12). Children who started wheezing in early life and continued to wheeze at the age of six ("persistent wheezers") were more likely to have normal lung function in the first year of life, and diminished values for *V'*max FRC at six years of age (12).

In the Manchester Asthma and Allergy Study birth cohort (62), 69 high risk infants (both parents atopic) underwent examination with the partial forced expiratory flow volume technique to determine *V'*max FRC. In this study, similar to findings from the Tucson study, authors found significantly lower *V'*max FRC in infants who had recurrent wheeze during the first year of life than in those who did not. In another birth cohort study from Perth (63), partial forced expiratory flow-volume curves obtained by rapid thoracic compression technique were assessed in 253 infants. In this study, the authors found that wheezing that begins or persists into the second year of life is usually associated with diminished lung function while wheezing during the first year of life is often a transient condition which improves with time (63).

Carlsen et al (65) from the ECA birth cohort study assessed whether tidal flow patterns can be used to discriminate between children with asthma and those without respiratory illness and whether reversibility to salbutamol in young children can be detected by tidal breathing analysis. They found that the ratios of the time and volume until peak expiratory flow to the total expiratory time and volume, and the ratio of tidal expiratory flow at 25% remaining expiration to peak expiratory flow, were significantly lower in asthmatic children than in controls, and increased significantly after salbutamol inhalation in asthmatic children (65).

Based on the results from prospective longitudinal studies (53;61-63;65), there is substantial evidence to suggest that the changes in lung function in children with asthma and asthma-like symptoms occur as early as first year of life or may be present before the first respiratory

illness. It is also supported by our recent report (66) which has shown the association between reduced lung function at birth and increased risk of asthma at 10 years of age.

5.7 Childhood asthma and bronchial hyper responsiveness

Bronchial hyper responsiveness (BHR) is commonly seen in asthma and has a role in the pathophysiology of asthma (67). However, it can also be seen in asymptomatic subjects (68). It has been reported that BHR is already present in very young children (69).

To investigate whether airway responsiveness is present from birth or if it develops as a result of subsequent insults to the respiratory tract, Young and colleagues (70) assessed airway responsiveness in 63 normal infants at a mean age of 4 1/2 weeks. Airway responsiveness was assessed by histamine inhalation challenge and the provocation concentration of histamine resulting in a 40% fall on *V'*max FRC from baseline (PC40) was determined. In this study, airway responsiveness was increased in infants with a family history of asthma, parental smoking, or both, as compared with the infants with no family history of asthma or smoking (70). From the Tucson Children's Respiratory Study, Lombardi and colleagues (71) studied the relationship between BHR to dry, cold air at age six and the subsequent incidence of asthma. They found that BHR to cold air at age six was associated with an increased risk of developing subsequent asthma at age 11 after 5-year follow-up, but this effect was not independent of atopy and mild wheezing at age six (71).

In a cross-sectional study of 2363 Australian school children (72), BHR was associated with atopic status, a history of asthma in either parent or a history of early respiratory illness. Furthermore, sensitization to house dust mite and cat was significantly associated with BHR in Chinese school children (73).

The relationship between BHR and bronchodilator response is not well known. In a study of 7-16 year old Dutch children bronchodilator response was weakly related to BHR (74). Lødrup Carlsen and colleagues (75) studied the bronchodilator response in young children in

subjects included in a nested case-control study (children with rBO) and controls without a history of lower respiratory disease by two years of age. They found that the mean per cent change in time to reach peak flow/total expiratory time (t_{PTEF}/t_E) from before to after salbutamol was significantly higher in children with rBO compared to controls. The latter study (75), however did not measure BHR in relation to bronchodilator response.

5.8 Pathophysiology of asthmatic airways

Asthma is an inflammatory disorder of the airways, which involves several inflammatory cells and multiple mediators that result in characteristic pathophysiological changes (76). There is evidence to suggest that asthma acts via a chronic inflammatory process that causes remodelling of the airways with increased thickening of reticular layer of basement membrane and smooth muscle hypertrophy (76;77). The inflammation with increased number of lymphocytes are uniformly distributed in the large and small airways in mild or severe asthma (78). The pattern of inflammation in the airways appears to be similar in all clinical forms of asthma, whether allergic or non-allergic (76).

A study of bronchial biopsies in children aged 1.2-11.7 years by Pohunek and co-workers (79) found that eosinophilic inflammation and airway remodelling occur early in the natural history of bronchial asthma and are present even before asthma would be diagnosed based on clinical symptoms. However, Saglani and co-workers (80) working on endobronchial biopsies obtained from 53 infants (median age 12 months, range 3.4-26 months) during clinical bronchoscopy for severe wheeze and/or cough found no significant differences in reticular basement membrane thickness or eosinophilic inflammation in symptomatic infants with reversible airflow obstruction, even in the presence of atopy. They found that the reticular basement membrane thickness in all infants was significantly less thick than that in the older children with asthma (80).

The clinical spectrum of asthma is highly variable but the presence of airway inflammation

remains a consistent feature (76). Therefore, the importance of controller medications with an effect on the underlying inflammatory process has been emphasized in the most recent treatment recommendations (3;81).

5.9 Asthma treatment in children

The aim of present international and national treatment guidelines is to control asthma by reducing underlying lung inflammation, improving symptoms and prevent exacerbations (3;81-83). Advances in our knowledge of the exact mechanisms of asthma disease continue to lead to better management options. Anti-asthma medications may be classified as symptom-modifying ('relievers', e.g. bronchodilators) or symptom preventers ('controllers', e.g. inhaled cortiocsteroids) (14). Symptom-modifying drugs act by relaxing smooth muscle and thus improving airflow through the conducting airways (3). Symptom preventing drugs prevent or reduce symptoms of asthma, generally by their effect on the airway inflammatory process (3).

A stepwise approach to pharmacological treatment to achieve and maintain control of asthma should take into account the safety of treatment, potential adverse effects, and the cost of treatment required to achieve control (3). For long term management of asthma in children, the standard classification of asthma severity from GINA (3) and the National Institutes of Health consensus guidelines (84) can be used. The guidelines for asthma management in adults differ from that of children and more so for younger children. Furthermore, the guidelines vary in different regions of the world based on local treatment traditions and documentation of effects. The specific medical treatment recommended to patients with asthma depends on the severity of their illness. In mild intermittent asthma (with symptoms 2 days/week or less), no daily controller medication is needed, however relievers can be used when needed. In mild persistent asthma (symptoms more than 2 times/week, but less than one/day), low dose ICS can be used. For moderate persistent asthma (daily symptoms during

day and > 1 night/week), low-dose ICS plus long acting ß-2 agonist or medium or high dose ICS or low dose ICS plus leukotriene modifier can be used. For severe persistent asthma (continuous symptoms during day time and frequent night symptoms), high dose ICS as well as long acting ß-2 agonist and or leukotriene modifier can be added. These treatment strategies are some of the existing guidelines for asthma management and not necessarily practiced everywhere. In addition to anti-asthma medications, education programs for caregivers and self-management training for children with asthma is emphasized.

As there is evidence supporting an apparent pathophysiologic relation between allergic rhinitis and asthma, subjects with both rhinitis and asthma benefit by treatment with systemic agents, such as antihistamines alone or in combination with leukotriene modifiers, or corticosteroids (85). Immunotherapy can be used as an adjunct to standard drug therapy in allergic asthmatic children (86).

5.10 Inhaled corticosteroids in childhood asthma

According to the latest revision of GINA guidelines (3), inhaled corticosteroids (ICS) are preferred as first line treatment for persistent asthma in children. ICS were introduced more than three decades ago, and they still are regarded as the most effective therapy available for asthma. The extent of use of ICS depends on the prevalence of asthma and local practice of asthma management. Limited information has been available about how often ICS are used for treatment of asthma-like symptoms in early life. A cross-sectional telephone survey (87) was conducted during 1996-7 to parental caretakers of 2-12-year-old children who had been hospitalized with asthma. In this study of children with symptoms of moderate to severe persistent asthma (84), only 15% received ICS in contrast to guidelines recommendations (87). Recently, Bisgaard et al (88) reporting from a cross-sectional telephone survey of child population (n=9490) aged 1-5 years in the USA and Europe found that 5% children with wheezing illnesses have used ICS as regular treatment (varied from 4% in Northern Europe

to 7% in Southern Europe).

In a large random sample (n=4,909) of Australian school children aged 8-11 years, 13 % reported use of ICS treatment during the period 1995-7 (89). In 1995-6 the use of ICS in the last 12 months was reported by 16 % of asthmatic German children (5-7 years and 9-11 years-old) (90). More recently a fourfold increase (from 2.5% in 1988 to 11.3% in 2001) in ICS use in the last 12 months was reported among 12 year-old British child population (91).

Effect on asthma symptoms, exacerbations, hyperresponsiveness

The anti-inflammatory effects of ICS are well established; inhibition of the production of cytokines, direct effect on eosinophils and neutrophils and decreased vascular permeability causing reduction of mucosal oedema (92). There is consistent evidence that ICS treatment in asthma improves the control of asthma symptoms (93) and reduces hospital admissions (93;94). Efficacy has also been shown by stopping medication, as patients frequently experience an exacerbation of their asthma upon withdrawal of ICS, as shown both in preschool and school children (95;96). A recent meta analysis report of management of acute worsenings and exacerbations of asthma showed consistent evidence of beneficial role of ICS in children (97).

ICS reduces BHR more effectively than other treatments (93). Koh et al (98) found significant improvement in BHR in children receiving ICS and the extent of improvement in children was found to be dependent on the degree of BHR in their parents. Nielsen et al. (99) in a randomized controlled trial (RCT) have demonstrated that inhaled budesonide at a total dose of 800 microgram daily significantly improved symptoms, asthma exacerbation rates, and BHR as assessed by cold air challenge in asthmatic children aged two to five years. Teper et al (100) in a RCT have shown that fluticasone treatment daily in children with recurrent wheezing increased percentage of symptom-free days, decreased number of exacerbations, and decreased percentage of days on short-acting bronchodilator.

Effects of ICS on lung function in children

In the last few years, the evidence is increasing regarding effects of ICS on lung function in preschool children (95;100;101). Teper and co-workers (100) in a double-blind placebo-controlled randomised study assessed the effects of treatment with fluticasone in children younger than two years with recurrent wheezing. In the latter study, subjects were assessed at the beginning and end of a 6-month treatment with fluticasone 125 μg two times daily or placebo. Lung function (*V'*max FRC) by the rapid thoracic-abdominal compression technique has improved in the group treated with fluticasone compared to group treated with placebo (100). Nielsen et al (99) in a RCT studied 38 asthmatic children aged two to five years (mean age 53 months) involving a eight week of 400 microgram twice daily budesonide treatment. Lung function measured as the specific airway resistance using whole-body plethysmography; as resistance by the interrupter technique (Rint); and as resistance and reactance by the impulse oscillation technique showed significant improvement in the budesonide treated group compared to placebo (99).

Several studies with different study designs have shown improved lung function in older children treated with ICS (94;102;103) whereas others did not (93). One non-randomized study (94) demonstrated that early treatment with inhaled budesonide after the diagnosis of asthma was established improved FEV_1 in older children (mean age 6.2 years) both compared with the run-in period and with the control group. In patients not treated with budesonide an annual decrease in % predicted FEV_1 of 1-3% was seen. After 3 years of treatment with budesonide, children who started therapy later than 5 years after the onset of asthma had significantly lower FEV_1 than the children who received budesonide within the first 2 years after the onset of asthma (94). An uncontrolled retrospective study (102) demonstrated significantly lower spirometric values in children (mean age 6.5 years) treated with bronchodilators alone, compared to those receiving anti-inflammatory treatment with

ICS or disodium cromoglycate. In a Dutch randomized double-blind multicentre study (104), asthmatic children treated with inhaled budesonide showed increased FEV_1 % predicted, post-bronchodilator FEV_1 and increased peak expiratory flow rate when compared to placebo, which was then maintained for a median follow-up time of 22 months. Similarly, increase in FEV_1 and improved BHR were seen in asthmatic children treated with inhaled budesonide in the study by Kerrebijn et al (105). Also Merkus et al (106) reported improved lung function (assessed as change of FEV_1 and of maximal expiratory flows) after use of ICS in school children as compared to placebo. Pauwels and colleagues (107) have studied the benefits of early intervention with inhaled budesonide, starting less than two years after the diagnosis of asthma. This study which also included 1,974 were children aged 5-10 years and have received either budesonide or placebo once daily for 3 years in addition to their usual asthma medications. Subjects on budesonide had increased postbronchodilator FEV_1 from baseline after 1 year and after 3 years as well as decreased risk of exacerbations and more symptom-free days than those receiving placebo (107).

On the other hand, RCT from the Childhood Asthma Management Program (CAMP) (93) failed to show improvement on the degree of change in the FEV_1 among 5-12 year old children treated with ICS for 4-6 years compared to placebo. In this study, 1041 children with mild to moderate asthma received either 200 microgram of budesonide twice daily or nedocromil or placebo (93). The enrolled subjects had asthma for a mean period of five years before they received treatment. However, the children given budesonide had lower airway responsiveness to methacholine and better control of asthma symptoms (93).

A Dutch study (96) in school children treated for 28-36 months with ICS showed symptomatic improvement only during the treatment period while experiencing recurring asthma symptoms after stopping ICS.

Effect of early ICS treatment on prognosis of asthma

Delayed introduction of ICS has been reported to result in reduced improvement in lung function compared with early use of inhaled steroids in childhood (94). Thus, a change in treatment strategy toward earlier introduction of ICS has been recommended. However, longitudinal studies in children are needed to clarify whether ICS has any effect on long term prognosis of asthma in children.

During the last year, two RCTs (95;101) in preschool children were undertaken to assess if ICS treatment does alter the natural course of asthma in early childhood. However, these studies failed to show improved lung function after cessation of ICS treatment (95;101). In a double blind RCT by Guilbert and colleagues (95) studied whether treatment with fluticasone propionate can modify the subsequent development of asthma in preschool children (n=285) at high risk (positive asthma predictive index) for asthma. One group of children received two puffs of fluticasone (44 µg per puff) twice daily and the other group received placebo during the 24-month treatment period. Although there was initial improvement, there was no significant difference between the study groups in any measure derived from impulse oscillometry at the end of a third, treatment-free year (95). Authors concluded that two years of inhaled corticosteroid therapy did not change the development of asthma symptoms or lung function during a third, treatment-free year (95). Similar findings were observed in a double-blind RCT by Murray and co-workers (101). In this study, the effect of inhaled fluticasone propionate 100 microgram twice daily in young children (median age 1.2 years) who were followed prospectively and randomised after either one prolonged (>1 month) or two medically confirmed wheezy episodes. Children in the study had treatment for varying periods depending on symptom control (minimum 9 months, but could continue on treatment up to fifth birthday). Children were followed-up to five years of age, at which point children's lung function (specific airways resistance, forced expiratory volume in one second and

airway reactivity) was assessed. They found that the groups did not differ significantly with regards to current wheeze, physician-diagnosed asthma, lung function or airway reactivity (101). However, there was significant improvement in symptom scores and the number of unscheduled physician visits for children in the treatment group, but only during the third month of the study.

6 AIMS OF THE THESIS

Main objective

The main objective of the present thesis was to assess if early treatment with inhaled corticosteroids could modify disease progression in childhood.

To study the main objective, it is of interest to know the prevalence of recurrent bronchial obstruction in early life and asthma in school age and assess how severity of the disease in early life can influence later occurrence of the disease. Secondly, the extent of use of inhaled corticosteroids in young children needs to be elucidated. Lastly, the purpose was to assess if ICS treatment has any effect on disease progression with relation to lung function by two years of age and occurrence of asthma by 10 years of age.

Specific aims:

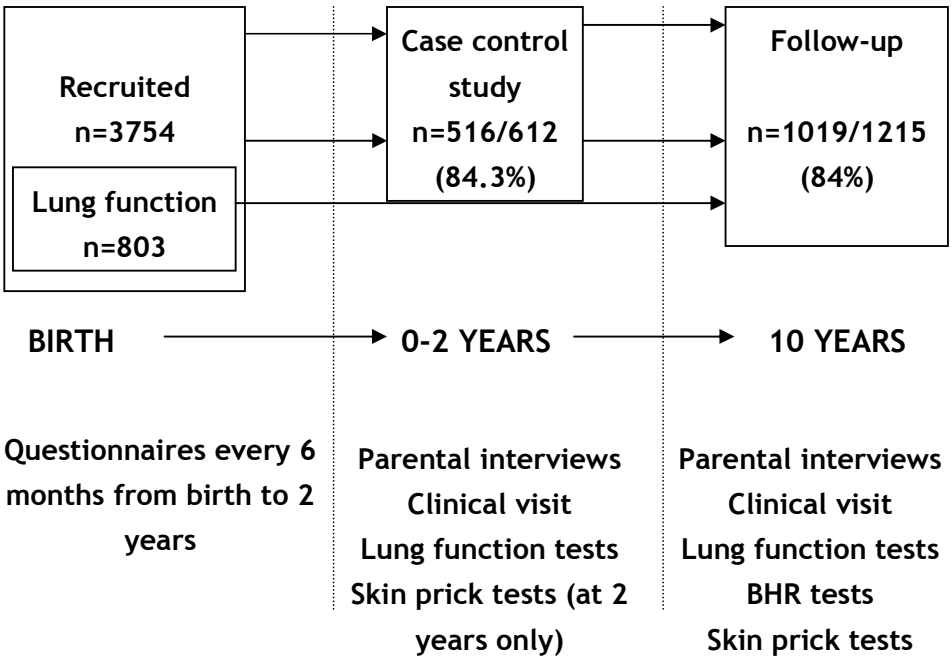
1. To investigate the prevalence of recurrent bronchial obstruction and asthma in children in a general urban population.
2. To determine how often inhaled corticosteroids were used for treatment of obstructive airways disease in childhood.
3. To assess if ICS use had an effect on lung function in young children with recurrent bronchial obstruction.
4. To define a severity score for severity of obstructive airways disease during the first two years of life and assess if the severity score can be used as a tool to predict asthma in school children.
5. To explore if early ICS treatment in children with obstructive airways disease during the first two years of age can modify occurrence of current asthma in school children.

7 STUDY DESIGN

Environment and Childhood Asthma study

The ECA study was established in 1992 with main purpose of identifying factors including environmental factors that were involved in the development of asthma in young children, as a collaborative study between Department of Paediatrics at Ullevål University Hospital and Section for Epidemiology at National Institute of Public Health.

The ECA study is a prospective birth cohort with 3,754 children enrolled at birth in Oslo during 15 months from January 1st1992. See figure 1 below on the study design and main methods of clinical part of the ECA study.



Study design and main methods of the ECA birth cohort study.

Inclusion criteria were:

- Babies born at Ullevål Hospital and Aker Hospital, Oslo.
- Birth weight of more than 2000 g.

- Absence of any illness likely to impair respiration (severe respiratory, cardiovascular, neuromuscular or metabolic disease).
- No requirement for assisted ventilation or oxygen therapy beyond six hours after birth.

Exclusion criteria were:

- Plans to move out of Oslo within six months.
- Insufficient language comprehension by the parents to be able to complete questionnaires.

Lung function measurements (tidal flow-volume loops and passive respiratory mechanics) were performed shortly after birth in 803 healthy newborn children (108).

Nested case control study

A nested case-control study was established during the first two years of life to perform detailed examinations of children with recurrent bronchial obstruction (rBO) (n=306) and age-matched controls (n=306). *Case* was defined as a child with recurrent (more than one) or persistent (more than four weeks) doctor confirmed bronchial obstruction (BO) by two years of age. *Control* was defined as a child without symptoms of lower respiratory illness born closest in time to the case.

The following recruitment procedures were performed to identify possible children with rBO (cases) for the nested case-control study:

- All questionnaires and parental interview forms were scrutinized for possible symptoms of recurrent BO.
- Clinical signs related to BO were recorded on a check-box card by all doctors examining a participating child during a respiratory tract illness, and the cards were returned to the study personnel at least every 6 months for identification of a possible case.

- All family doctors, well baby clinics, accident and emergency departments and paediatric hospital wards were requested to refer participating children with possible or established BO to the study paediatrician.

Any of the above events led to a request to attend a clinical examination by the study paediatrician for eligibility to enrol into the nested case-control study. All children were asked to attend one or two visits (if older or younger than 20 months, respectively, at enrolment in the case-control study). The first clinical visit was performed as soon as possible after eligibility was established at a mean age of 11 months, and the second clinical visit at a mean age of 25 months. The attendance rate at the two-year visit was 84 %, almost equally distributed between cases (n = 265) and controls (n = 251).

The nested case control study was performed in the absence of clinical signs of bronchial obstruction or upper respiratory infection, and consisted of a structured interview (detailed medical and allergic family history, pet keeping and tobacco smoke exposure), clinical examination, lung function assessed by tidal flow volume loops including ßeta-2 responsiveness, skin prick test to food and common inhalant allergens, serum for markers of inflammation, immunoglobulins (Ig), and total IgE, as well as environmental exposure assessments (indoor and outdoor).

10 year follow-up study

To understand better the early risk factors for later asthma and allergy development in school children, a follow-up study of children with lung function measured at birth (*n* = 803) and/or a clinical investigation by 2 years of age, was initiated in 2001 (see figure 1 above).

A total of 1215 children aged 9-10 years were invited. Of these, 1019 (84 %) attended the two-day follow-up study between September 2001 and July 2004. The study included detailed parental structured interview, blood tests, skin prick tests (SPT), spirometry,

methacholine challenge test and clinical examinations on day 1, and an exercise test by treadmill running within 1 week.

Other clinical examinations included exhaled nitric oxide measurements, urine sampling, markers of airway inflammation, genetic analyses, and environmental exposure tests, which are not reported in the studies included in the present thesis.

8 METHODS

8.1 Parental questionnaires

All children were followed from birth until their second birthday half-yearly with questionnaires completed by the parents/guardians, for the first time at the maternity ward. Questionnaires included information on the child's health with specific questions related to respiratory, allergic, skin as well as general diseases and details about medical treatment including ICS, family history of atopic or other diseases, and parental smoking habits. Questions also included infectious diseases, socio-economic factors and environmental exposures. Of 3754 enrolled children, 3697 subjects also had a completed two year follow-up questionnaire.

8.2 Parental interviews

At inclusion and 2 years

Furthermore, both at the first clinical visit at debut of OAD (mean age 11 months, range 3-21 months) and at the two year clinical visit (mean age 26.8 months, range 2-41 months), a parental structured interview by the study doctor was completed regarding diseases of index child and primary family members, infections, environmental exposures, housing, socio-economic factors and any type of medical treatment.

The following questions regarding treatment of rBO until two years of age were asked: "the use of preventive or regular therapy for wheezing?", if yes, "the use of ICS as regular therapy?" if yes, questions regarding age at start, duration and age at cessation of ICS therapy were asked.

The information regarding severity of obstructive airway symptoms until two years of age was assessed by the following questions: "has the child ever had wheezing and/or shortness of birth?", if yes, "the number of episodes with wheezing" and/or "the number of months

with persistent wheezing" and "was the child ever admitted to hospital due to wheezing?" and if yes, "the number of hospital admissions for wheezing". Answers to these questions were the basis of calculating severity score of OAD by two years of age (see the section on severity score).

At 10 years

Parental interviews were conducted by study physicians where parents of the enrolled children answered a structured, extensive questionnaire regarding housing, socio-economic factors, infections, diseases of index child and primary family members. The central core questions from International Study of Asthma and Allergies in Children (ISAAC) validated in Norwegian (109) were asked specially in relation to airways symptoms, medical treatment, tobacco exposure, physical activity and diet.

For defining *asthma ever (history of asthma)* at 10 years of age, the following questions were asked:

"Has your child experienced dyspnoea, chest tightness and/or wheezing during the age periods 0-3 years and/or 4-10 years?"

"Has the child been diagnosed having asthma by a doctor?" if "yes", "age at onset".

"Has the child ever taken medicine for asthma 0-3 years and/or 4-10 years??" if "yes", "what type of medicine" and "age at start of therapy".

As a part of defining *current asthma* at 10 years of age, the following questions were asked:

"Has your child experienced dyspnoea, chest tightness and/or wheezing during the last 12 months?"

"Has the child taken medicine for asthma during the last 12 months?" if "yes", "what type of medicine".

8.3 Lung function measurements

From birth to two years of age

Lung function was measured in 803 children shortly after birth (108) at a mean age 2.7 days by tidal flow volume (TFV) loops (n=802) and respiratory mechanics by the passive occlusion technique (n=664). All children were healthy at the time of lung function measurements.

Lung function was measured in 106 children included in the nested case-control study shortly after presentation of OAD at a mean age of 11 months. Lung function was also measured at the two year clinical visit in 312 children (264 with rBO and 148 controls) participating in the nested case control study.

Measurements of TFV loops using SensorMedics 2600 system (Anaheim, California) were performed by trained investigators and attempted in all subjects awake and quietly breathing (108;110). Among rBO children who were using medications, short-acting bronchodilators were withheld on the morning prior to testing (none of the children had received long-acting bronchodilators). TFV loops were obtained with a face mask (Vital Signs inc.) connected to pneumotachograph (4500 series, Hans Rudolph, Missouri, USA) with a flow range of 0-30 l/min. Dead-space of the system was 2.4 ml, and of the face mask was 8.4-11 ml. Volume was derived by the digital integration of the flow signal, which occurred at a sampling frequency of 256 samples per second without any filtering of the raw signal. Calibration of the flow and volume signals was performed daily, using a 100 ml precision syringe (Hans Rudolph).

Four representative TFV loops were stored for the final analysis. Each loop was chosen from eight stored loops obtained from series of breaths during established tidal breathing. The loops were selected from tidal breaths with as stable volume and shape of the loops as possible, and the respiratory rate being as low as possible. The ratio of time until peak tidal expiratory flow to total expiratory time (t_{PTEF}/t_E) was calculated by separate measurements of the time to peak tidal expiratory flow and total expiratory time by the software programme

of the computer (108;111).

The shapes of TFV loops represented by t_{PTEF}/t_E are reported to reflect OAD in children (111-115), as well as predict wheeze in children under three years of age (115). Based on this information, in paper II, we have used t_{PTEF}/t_E to assess possible effects of ICS treatment on evolution of lung function in children with rBO.

Bronchodilator responsiveness

Bronchodilator responsiveness was assessed by TFV measurement immediately before and 15 minutes after inhaled, nebulized salbutamol 0.05 mg/kg bodyweight (111). The subjects were classified as responders to salbutamol when the increase in mean t_{PTEF}/t_E after inhalation of salbutamol exceeded 2 SD of the intrasubject variation. Non-responders had a difference in mean t_{PTEF}/t_E before to after salbutamol inhalation of less than two SD of the initial intrasubject variation.

At 10 years

Maximum forced expiratory flow volume loops were measured according to European standard (116) (reference values of Zapletal (117)) on a SensorMedics Vmax 20c (SensorMedics Diagnostics, Yorba Linda, CA, USA) on four occasions; prior to metacholine and exercise challenge tests and after salbutamol given at the end of challenge tests, respectively (on separate days). Lung function measures were forced expiratory volume in one second (FEV_1) % predicted, forced expiratory flow at 50 % of vital capacity (FEF_{50}) % predicted and forced vital capacity (FVC) % predicted. The reported values in paper I were the best baseline values obtained before challenge testing.

8.4 Bronchial hyper responsiveness tests

BHR tests were performed at 10 year follow-up visit. Methacholine challenge test was performed the first day and exercise challenge (treadmill run) within one week after

withholding short and long acting ß-2 agonists for at least 12 and 48 hours respectively, and leukotriene antagonists for 72 hours.

Methacholine provocation test

BHR by methacholine provocation was measured according to international guidelines (118) by inhalation of doubling doses of methacholine, nebulised by the Spira nebuliser (Spira Respiratory Care Center Ltd, Håmeenlinna, Finland), until reaching a cumulated dose of methacholine causing a reduction in forced expiratory volume in 1 second (FEV_1) of 20 % (PD20) methacholine) or reaching the maximum cumulated dose (PD20) of 22.4 µmol methacholine.

Exercise provocation (treadmill run) test

A standardized exercise test (119) was performed by a 6-8 minute treadmill run, of which the last four minutes at 95 % maximal heart rate with 5.5 % inclination, with a 20 minute observation time after running. FEV_1 was measured before, and three, six, ten and 15 minutes after running as well as 10 minutes after inhalation of nebulised salbutamol (0.1 ml per 10 kg bodyweight) administered 20 minutes after running. The exercise challenge test was considered positive with ≥10 % reduction of baseline FEV_1 3-20 minutes after running ceased.

8.5 Skin Prick Tests

At 2 years

Skin Prick tests (SPT) were performed in 498 children at the two year visit, according to Nordic standard (120). The following standardised extracts from ALK (Hørsholm, Denmark) were used: egg white, cows milk, dermatophagoides (D.) pteronyssinus, timothy grass, cat dander, dog dander, silver birch, mugwort and cladosporium herbarum, as well as saline negative control and histamine control (10 mg/ml). Sensitization was regarded as positive with a wheal for the allergen in question of at least half the size of the wheal for the

histamine positive control.

At 10 years

SPT to common inhalant and food allergens were performed in 1010 children attending 10 year follow-up visit with Soluprick® allergens (ALK Albello, Denmark). Sensitization was considered positive with a wheal diameter ≥ 3mm larger than the negative control (NaCl). The following allergens were used: domestic mites (Dermatophagoides (D.) pteronnysinus and D. farinae), German cockroach, dog, cat, and rabbit dander, birch, timothy (grass) and mugwort pollens, moulds (Cladosporium herbarium and Alternaria alternata), egg white, milk, peanut and codfish.

8.6 Severity score

As reported in paper III, a severity score for each individual subject was calculated based upon the clinical criteria present during 0-2 years of age: number of episodes of bronchial obstruction, number of months with persistent bronchial obstruction and number of hospital admissions due to bronchial obstruction (see figure below).

BO episodes and/or persisting BO	Points
None	0
1-2 episodes or 1 month persisting	1
3-4 episodes	2
2-3 months persisting	3
5-6 episodes	4
4-6 months persisting	5
>6 episodes or >6 months persisting	6

+

No. of hospital adm. for BO	Points
None	0
1	2
2	4
>2	6

= **Severity Score (0-12)**

Figure 2 (figure 2 from paper III): Criteria used to define severity score. Reprinted with the permission from BMJ Publishing group Ltd, United Kongdom.

The information was obtained from questionnaires completed by the parents every six months and parental interview forms at debut visit and at the two year visit. Severity of OAD by two years of age was assessed by the questions previously described in the section about parental interviews.

Minimum and maximum score for an individual was 0 and 12, respectively. Two no-BO subjects had severity score of one.

The severity score was also calculated for the subjects at the age of 12 months, based on questionnaire data obtained every six months from birth to one year of age. The score at 12 months was constructed from data obtained in questionnaires only, with less detailed information than that obtained during parental interviews at the two year visit.

8.7 Statistical methods

General considerations

The outcomes were compared between the groups and differences were assessed for statistical significance, provided normal distribution of the data in question, between unpaired groups by using the unpaired (two-sample) t-test, and comparisons between paired groups with paired (one-sample) t-test. If data did not satisfy normal distribution, the non-parametric Mann-Whitney or Wilcoxon signed rank test were used, respectively. Analysis of variance tests were used for comparing multiple groups. Correlation analyses were performed with Pearson's correlation, using Fishers Z-transformation to obtain two-tailed test for significance. Lung function values were given as mean values with 95 % confidence intervals (CI).

Receiver operated characteristic analysis

Receiver operating characteristic (ROC) curves are used to assess the accuracy of a diagnostic test (121). The technique is used when one has a criterion variable which will be used to make a yes or no decision based on the value of this variable. The ROC curve is a graphical approach to plot the sensitivity versus 1-specificity for each possible cut-off, and to join the points (121). In paper III, ROC curve analysis was done by using the severity score 1-12 at two years as a continuous variable and classification by current asthma at ten years of age as clinical outcome. Sensitivity (%), specificity (%), positive predictive value (PPV) (%)

and negative predictive values (NPV) (%) was calculated for each value of the severity score (1-12) and reported in table 4 of paper III.

Logistic regression

The risk of current asthma at the age of 10 years was assessed using logistic regression analysis comparing rBO children with severity score of above five and no BO subjects (paper III). Odds ratios (OR) including 95% CI were calculated after adjustment for gender, parental atopy and atopic eczema by two years of age as well as allergic skin sensitization at two year visit. The appropriateness of the logistic model was assessed using Hosmer and Lemeshow Goodness of fit statistic for the overall fit of the model and Dfbetas and Cbar statistics to assess the influence of the single subjects (122). Linearity of severity score as a continuous variable was assessed using Hosmer's procedure (122).

The prediction models of loose and stringent indices suggested by Castro-Rodriguez et al (58) were approximated to similar data in paper III. The data that was used in the paper by Castro-Rodriguez et al (58) regarding frequency of wheezing the first three years of life, history of eczema, parental history of asthma, eosinophilia, and wheezing without colds was also found in our questionnaires and parental interviews between 0-2 years of age. However, we did not have comparable data regarding allergic rhinitis between 0-2 years of age (that is in our view difficult to assess in this age group) in our study and therefore data used to calculate asthma indices suggested by Castro-Rodriguez et al (58) was approximated. Sensitivity, specificity, positive and negative predictive values for both loose and stringent indices were calculated and presented in table 4 of paper III.

Propensity modelling and propensity score

Logistic regression by propensity modelling was used in paper IV to assess the risk of current asthma among rBO children with and without early ICS treatment compared to no-BO children. The propensity score has been proposed as a method of adjusting for bias in

treatment assignment present in observational studies (123) and as such being recommended in a paper from the Food and Drug Administration in the USA to correct for non-randomness (124). Since several factors that were expected to be associated with the use of ICS by two years could also be expected to affect later prognosis of the disease, a propensity model (125) was developed to adjust for such factors. A common approach is to include various predictors of outcome. The propensity model included gender, severity score by two years, and atopic dermatitis by two years as covariates. Furthermore, parental atopy and allergic skin sensitization by two years of age were assessed as possible confounders; however, they were not included in the propensity model due to questionable model fit. The propensity method used in paper IV is a logistic regression analysis with the use of ICS, as the dependent variable. The propensity scores are the predicted probabilities for using ICS for each individual. To assess the characteristics of the propensity scores, mean values were calculated for two different groups (ICS-treated and ICS-naïve children) of subjects. In the final logistic regression analysis the propensity score was used as a covariate together with the use of ICS treatment.

Regression to the mean

The potential risk of regression to the mean as described by Altman (126) was assessed in paper II in different treatment groups (ICS-treated, ICS-nontreated and controls). The solution to this problem as described by Altman (126) is to take the average of initial (X) and final measurement (Y) and calculate the correlation between this quantity and the observed change: $(X + Y)/2$ with $X–Y$.

9 STUDY SUBJECTS

Aim 1

"Investigate the prevalence of recurrent bronchial obstruction and asthma in children in a general urban population".

For the purpose of investigating the extent of prevalence of rBO by two years of age, the study included all children from the entire cohort who had completed all four follow-up questionnaires (3697 of 3754 subjects) as well as all children defined with rBO who had attended at least one visit.

To assess the prevalence of asthma until 10 years of age, the study included the 616 of 803 children with lung function measurements shortly after birth who attended the 10 year examination at a mean age (standard deviation) of 10.9 (0.9) years. Sufficient information was available to classify 614 children as having a history of asthma or not and 606 as having current asthma or not. The included children were representative of the entire birth cohort (n=3754) as there were no significant differences at birth with respect to gender, parental age, parental asthma, eczema or rhinoconjunctivitis, maternal smoking during pregnancy, pet keeping, parental education, paternal employment rate, family income or number of siblings (table 1, paper I).

Aim 2

"Determine how often inhaled corticosteroids were used for treatment of obstructive airways disease in early childhood."

The study included all children from the entire cohort who had completed all five questionnaires (3697 of 3754 subjects) up to two years of age as well as all children defined with rBO who had attended at least one visit.

Aim 3

"Assess if ICS treatment had an effect on lung function in young children with rBO."

For this aim, the 69 children from the case control study (54 cases and 15 controls) up to two years of age in whom lung function measurements had been obtained on two occasions were investigated. ICS had been used by 21 (ICS+) of the 54 cases (39%) and none of the controls. All children were steroid naïve at the first study visit and 79% remained so until two years of age (ICS-). Mean (SD) duration of treatment with ICS was 10.3 (6.5) months.

Of all the children participating in the case control study (n =516), 59 rBO children received ICS during the first two years of life, and 32 of these were still using ICS at the two year visit. None of the control children used ICS during the first two years of life.

A group of children used only for assessing the representativity of the included children (54 cases and 15 controls), comprised of remaining 243 children (110 with rBO and 133 controls) who had lung function measurements obtained at visit 2 only and the remaining 37 children (19 with rBO and 18 controls) with lung function measurements obtained at visit 1 only. The 69 included children did not differ significantly from the remaining non-included children with respect to demographic data (sex, weight, height, positive SPT, daily maternal smoking or parental atopic disease) whereas age was significantly lower (p=0.004) among the included children.

Aim 4

"Define a severity score of OAD in the first two years of life and assess the usefulness in predicting asthma in school children."

To define a severity score, we gathered information from subjects having recurrent bronchial obstruction (also defined as 'case' by two years of age) and compared with those subjects without BO (also defined as 'control' by two years of age). The study focused on clinical presentation at the time of investigation (two and 10 years) to assess if severity score by two years can predict asthma at 10 years of age. For this purpose, the subjects from the nested case control study who attended both the two year and 10 year visits (follow-up rate of 89%)

comprising 239 rBO subjects and 220 no-BO subjects, were investigated (see figure below). Mean age (SD) at the 10 year visit was 10.3 (0.7) years. However, due to insufficient data at two and/or 10 years in 10 children, the present study included the 449 children (233 rBO subjects and 216 no-BO subjects) with complete data to calculate severity score at two years of age and current asthma at 10 years of age.

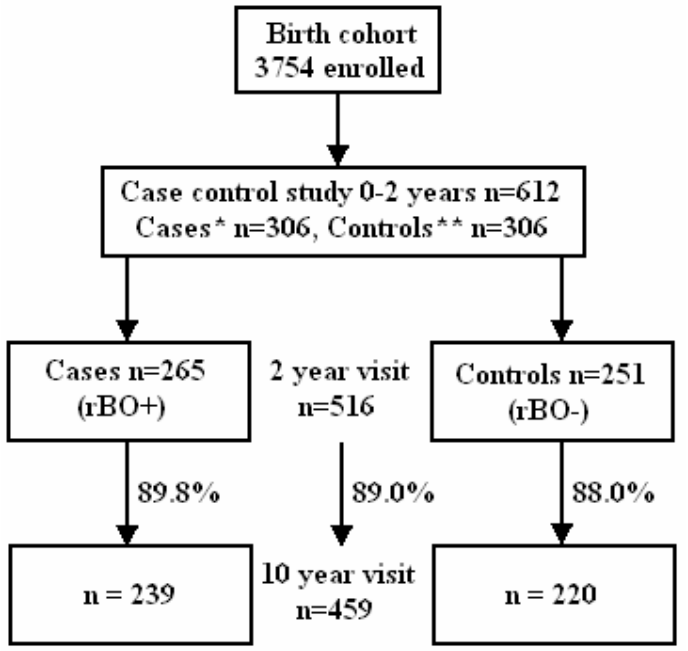


Figure 3 (Figure 1 from paper III). Flow chart of the study subjects. *Children with rBO were defined as children with recurrent bronchial obstruction by two years of age. Control** (rBO-): children were: age-matched children without bronchial obstruction by two years of age. Reprinted with the permission from BMJ Publishing group Ltd, United Kongdom.

Aim 5

"Explore if early ICS treatment in children with obstructive airways disease in the first two years of life can modify occurrence of current asthma in school children."

Similar to the study in paper III, we gathered information from subjects having obstructive airway symptoms and compared with those subjects without obstructive airway symptoms by two years of age. Additionally, the information on ICS treatment by two years of age in the

subjects was sought. As in paper III, the subjects from the nested case control study who attended both the two year and 10 year visits comprising 239 rBO subjects and 220 no-BO subjects were assessed. Of those, 452 had sufficient data to assess current asthma at 10 years of age. Mean age (SD) at the 10 year visit was 10.3 (0.7) years. Fifty one (21.9 %) rBO children reported use of ICS between 0-2 years of age, whereas the remaining 182 rBO children and none of the control subjects received ICS during these years. Mean (SD) duration of ICS therapy until two years was 8.5 (5.9) months (range 1-22 months).

10 OUTCOMES

For the aim of investigating the prevalence of asthma and current asthma at 10 years of age following outcomes were used (paper I):

Asthma was defined by at least two of the following three criteria being fulfilled:

1. Dyspnoea, chest tightness and/or wheezing 0-3 years and/or 4-10 years.
2. Physician's diagnosis of asthma.
3. Use of asthma medication (ß-2 agonist, sodium cromoglycate, corticosteroids, leukotriene antagonists and/or aminophylline) 0-3 years and/or 4-10 years.

Current asthma was defined as asthma (by definition above) plus at least one of the following:

1. Dyspnoea, chest tightness and/or wheezing during the last 12 months.
2. Use of asthma medication (ß-2 agonist, sodium cromoglycate, corticosteroids, leukotriene antagonists and/or aminophylline) during the last 12 months.
3. Positive exercise test.

Wheeze ever was defined by a positive response to the questions "Has your child experienced dyspnoea, chest tightness and/or wheezing during the age periods 0-3 years and/or 4-10 years?"

Lung function outcomes: baseline (pre-bronchodilaltor) FEV_1 % predicted and FEF_{50} % predicted.

For the aim of assessing the extent of use of ICS treatment in young children by two years of age, the outcome was subjects who received ICS treatment during the first two years of life (paper II). To assess if ICS treatment had an effect on lung function

in young children with rBO, the main outcome measure was t_{PTEF}/t_E ratios derived from TFV loops measured at debut visit (visit 1) and visit 2 (two year visit). Baseline t_{PTEF}/t_E (pre-bronchodilator) and post-bronchodilator t_{PTEF}/t_E ratios were calculated at both visits (paper II).

The secondary outcome was bronchodilator responsiveness expressed as change in t_{PTEF}/t_E from before to after salbutamol inhalation given by the formula: (t_{PTEF}/t_E after salbutamol – baseline t_{PTEF}/t_E) x baseline t_{PTEF}/t_E $^{-}1$, calculated at both visits (paper II).

For the aim of defining a severity score of obstructive airways disease in the first two years of life and assess if the severity score can be used as a tool to predict asthma in school children following outcomes were used: the primary outcome was current asthma at 10 years of age and the secondary outcome was non-specific BHR to methacholine (PD_{20}) (paper III).

For the aim of exploring if early ICS treatment in children with obstructive airways disease at two years of age can modify occurrence of current asthma in school children, the main outcome was current asthma at 10 years of age (paper IV).

Definitions:

Allergic skin sensitization

Allergic skin sensitization reported in papers I, II, III and IV were considered positive with ≥ one positive skin prick test to common inhalant and/or food allergens.

Parental atopy

Parental atopy reported in papers II, III and IV was defined as asthma and/or rhinoconjunctivitis reported in at least one parent by two years of age.

11 ETHICAL CONSIDERATIONS

Parents of all eligible children received written information at each phase of the study. Signed, informed consent was obtained from parents of all subjects upon enrolment. The study was approved by the Regional Committee for Medical Ethics and the Norwegian Data Inspectorate. Applications for renewed approval were obtained for the 10 year follow up study. The study was registered in the Norwegian Biobank Registry, Ullevål University Hospital in Oslo.

12 MAIN RESULTS OF THE PRESENT STUDIES

12.1 Prevalence of recurrent bronchial obstruction or asthma and allergic sensitization in childhood

Recurrent bronchial obstruction in 0-2 year old children

Of all healthy children enrolled at birth in the study (3697 of 3754 with complete questionnaire data by two years of age), 306 subjects had documented symptoms of recurrent bronchial obstruction by two years of age, corresponding to prevalence of 8.3 % with rBO in young children (paper III).

Asthma until 10 years of age

At the 10 year follow-up visit, lifetime prevalence of asthma was 20.2 %, current asthma 11.1 %, doctors' diagnosis of asthma 16.1 %, and 30.6 % of the school children suffered from wheeze ever (paper I). Boys had more often than girls current asthma (14.4 vs. 7.1 %, respectively, p=0.008) and wheeze ever (36.9 % vs. 22.5 %, respectively, p=0.002). Wheeze was reported by 8.8% of subjects during the first three years of age only, compared to 9.2 % during 4-10 years of age only. Of 21.4 % children who wheezed during the first three years of age, 41.2% reported no later wheeze (paper I).

Lung function in asthmatic children at 10 years

At the 10 years follow-up visit, lung function as FEV_1 % predicted (mean, 95% CI) was significantly reduced among children with current asthma (95.8, 93.3-98.3) compared to asthma without current symptoms (100.3, 97.7-102.8) and no asthma (99.1, 98.3-100.0) (p = 0.021). The corresponding values for FEF_{50} % predicted were 79.0, 73.8-84.0 for current asthma, 86.7, 82.1-92.1 for asthma without current symptoms and 91.0, 89.4-92.7 for subjects without asthma, respectively, ($p<0.001$ for trend).

Allergic skin sensitization in asthmatic children at 10 years

At the 10 year visit, positive SPT to at least one allergen was found among 29.3% of all

children, significantly more frequent among children with current asthma (56.1%) compared to asthmatic children without current symptoms (26.8%) and no asthma (26.0%) ($p<0.001$). Children who wheezed during 4-10 years of age were more often sensitized than children who had never wheezed or wheezed only during the first three years of age. Boys more often than girls were sensitized to at least one allergen (36.2% vs 26.1%) ($p<0.001$) (paper I).

Parental atopy in asthmatic children at 10 years

A parental history of asthma and/or rhinoconjunctivitis by 10 years of age was significantly more common among children with current asthma (67.2%) compared to asthmatic children without current symptoms (49.1%) and children without asthma (49.6%) ($p=0.024$). The effect was mainly due to parental asthma (29.9% in children with current asthma, 12.3% in children with previous asthma and 13.5 % with no asthma, $p=0.002$) (paper I).

12.2 Extent of use of inhaled corticosteroids in young children

From the entire cohort (3697 of 3754 with complete questionnaire data by two years of age), 77 children corresponding to a prevalence of 2.1 % and 64 of the 306 children (20.9 %) with rBO (20.9%) had received treatment with inhaled steroids by the age of two years (paper II). Mean (SD) duration of ICS treatment among the rBO children with repeated lung function measurements (n=21) was 10.3 (6.5) months up to the two year follow-up visit (paper II).

12.3 Effect of inhaled corticosteroids on lung function in young children

Visit 1

Baseline (prebronchodilator) t_{PTEF}/t_E was significantly lower among children with rBO compared to controls at the inclusion visit (table 2, paper II). Furthermore, the mean (95% CI) baseline t_{PTEF}/t_E at the first visit was significantly lower in rBO children who later received ICS compared to those who did not (0.17, 0.15-0.20) versus 0.23, 0.19-0.28)) ($p=0.03$). Analysis of baseline t_{PTEF}/t_E between all the children who had received ICS before

two years of age with lung function measurements (n=39) compared to the corresponding rBO children who had never received ICS (n=125) confirmed the statistically significant differences with a mean difference in t_{PTEF}/t_E in favour of the ICS-naive children (0.07 0.03-0.12) (p=0.003).

Visit 2

Baseline t_{PTEF}/t_E (prebronchodilator) was also significantly lower among children with rBO compared to controls (table 2, paper II) whereas it was not significantly different between the treatment groups (ICS-treated and steroid-naive) at two years of age. At visit 2, baseline t_{PTEF}/t_E did not differ significantly between all the children who had received ICS by two years with lung function measurements (n=39) compared to the corresponding children who had never received ICS (n=125) (p=0.2)).

Baseline lung function did not differ significantly among the included 69 children as compared to the children with lung function measurements on one occasion only, neither at visit 1 (0.24, 0.21-0.27 versus 0.25, 0.21-0.29, respectively) nor at visit 2 (0.27, 0.25-0.30 versus 0.28, 0.27-0.29, respectively).

Change in lung function from visit 1 to visit 2

The mean difference in baseline t_{PTEF}/t_E from first to second visit was significantly higher (borderline) in the ICS-treated group only (0.057, -0.003-0.116) (p=0.06) (figure 4) and correlated significantly with duration of ICS treatment (r=0.481, p=0.027).

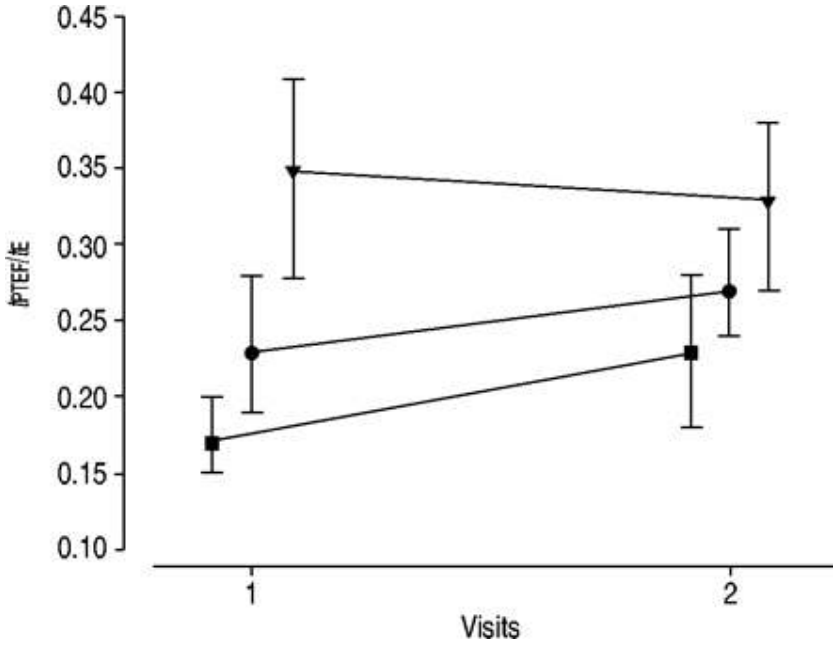


Fig. 4 (figure 2 from paper II).—Baseline lung function in 54 children with recurrent bronchial obstruction and controls (▼) at enrolment in case-control study and at 2 yrs (visit 1 and 2, respectively). Lung function represented by the ratio of time to peak expiratory flow to total expiratory time (t_{PTEF}/t_E) was significantly lower among children who later received inhaled corticosteroids (ICS; ICS+: ■) compared to those who did not receive ICS (ICS–: •) at first visit (p=0.03), but not at second visit (p=0.12).

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Bronchodilator responsiveness

In children with rBO, bronchodilator responsiveness was significantly higher (p=0.02) as compared to controls at visit 1 but not at visit 2, but without statistically significant differences between ICS+ and ICS- children at either visit. However, postbronchodilator t_{PTEF}/t_E ratios were not significantly different between the ICS+, ICS- and control groups either at visit 1 or visit 2.

12.4 Impact of severity of obstructive airways disease by two years of age on asthma at 10 years of age

Severity score by two years of age

The mean (95 % CI) severity score of all rBO subjects at two years of age was 4.6 (4.2-5.0),

significantly higher among rBO children who developed current asthma, 5.5 (4.9-6.1) compared to rBO children without current asthma 4.0 (3.6-4.5) ($p<0.001$). The mean severity score at the age of 12 months among rBO+ subjects was 1.04 (0.81-1.27).

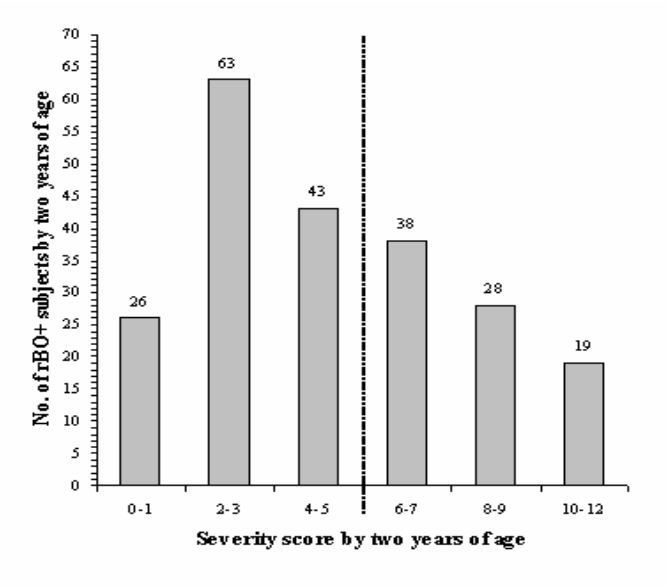

Figure 5 (figure 3 from Paper III). The severity score distribution at two years of age. Reprinted with the permission from BMJ Publishing group Ltd, United Kongdom.

Based upon the analysis of all included 449 children in the study reported in paper III, a linear association was found for severity score at two years of age as a predictor for current asthma at 10 years of age; the ORs (95% confidence intervals) for a one-point increase in severity score was 1.35 (1.25-1.45) and for a two-point increase in severity score 1.82 (1.57-2.11) respectively. By using the severity score calculated at the age 12 months the OR for current asthma at 10 years was 1.30 (1.11-1.52) for a one point increase in severity score.

Receiver operated characteristic analysis

The ROC analysis for severity score at two years as a continuous variable classified by current asthma at ten years, revealed an area under the curve of 0.78 (95% C.I.: 0.73, 0.83, $p<0.0001$) (see figure below). In the analysis, the cut-off value six provided the best value to predict current asthma at 10 years of age (odds ratio 7.9 %, sensitivity 51.5 %, specificity

88.1 %, PPV 54.3 % and NPV 86.8 %) (table 4, paper III).

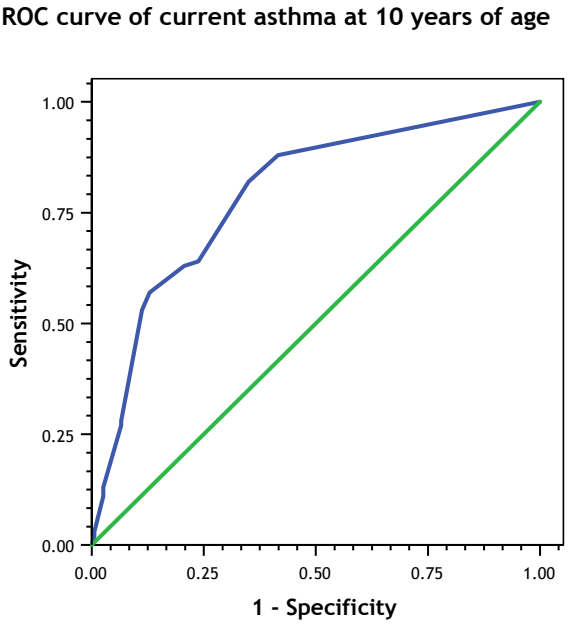

Figure 6. Severity score at two years of age and the ROC curve of current asthma at 10 years of age.

Adjusted OR and the results of the ROC analysis are shown in table 4 of paper III for each level of severity score with sensitivity, specificity, positive and negative predictive values for current asthma.

Severity score at 12 months of age

The ROC analysis for severity score at 12 months, classified by current asthma at 10 years revealed an area under the curve of 0.59 (0.54-0.64) (p=0.007). However, with a cut off value of severity score at 12 months set to two (identified as the best value), the sensitivity was only 16.8% (9.9%-25.9%), the specificity 94.3% (91.3%-96.5%) with a positive predictive value of 44.4% and negative predictive value of 80.6%.

Current asthma at 10 years of age

Current asthma at 10 years was significantly more frequent among rBO children (36.5 %) compared to no-BO subjects (5.6 %) ($p<0.001$). More boys than girls had current asthma at ten years of age ($p=0.05$). The rBO children with current asthma at 10 years of age were more often hospitalised due to BO by two years of age ($p=0.01$) than no-BO children without current asthma at 10 years of age (table 3 paper III).

Risk of current asthma at 10 years of age

The adjusted OR (adjusted for gender, parental atopy and atopic eczema by two years of age as well as allergic skin sensitization at two year visit) for current asthma among all rBO children compared to no-BO children was 7.9 (4.1-15.3). Compared to no-BO children, the adjusted OR for current asthma was 20.2 (9.9-41.3) for rBO children with severity score of 6-12 at two years of age.

Severity score versus asthma indices

The predictive capacities for current asthma at 10 years of age (compared to no-BO children) assessed by the stringent and loose asthma indices reported by Castro-Rodriguez et al (8) were applied to comparable data in the present study (table 4, paper III). Using a cut off value six in the present severity score at two years of age gave a higher positive predictive value (54.3 %), but similar negative predictive value (86.8 %) compared with the applied loose (87.7 %) and stringent indices (87.4 %) (table 4, paper III).

Bronchial hyper responsiveness

Children with higher severity scores above five at two years of age had significantly more often severe BHR (PD_{20}-methacholine < 1 μmol) compared to subjects with no severity score (22.2 % versus 8.5 %, respectively, $p=0.0041$). On the other hand, subjects with severity score above five had significantly less often mild or no BHR (PD_{20}methacholine > 8 μmol) compared to children with severity score 0 (44.5 % versus 60.2 %, respectively) (table 5,

paper III).

12.5 Impact of inhaled corticosteroids treatment by two years of age on asthma at 10 years of age

Current asthma at 10 years was significantly more frequent among ICS-treated children (56.9 %) compared to rBO children who were not ICS-treated (30.8 %) and no BO children (5.5 %) ($p<0.001$). Among children with current asthma, the rBO children with ICS treatment by two years of age versus those without had at two years significantly higher severity scores and had significantly more often been admitted to hospital due to BO (paper IV).

Risk of current asthma at 10 years of age

Analysis by logistic regression had demonstrated in rBO children that the male gender (adjusted OR (aOR): 1.82 (1.01 – 3.27), p=0.046) and severity score at the age of two years (aOR 1.14 (1.03 -1.28), p=0.014) were significant risk factors for current asthma at the 10 years, whereas the use of ICS treatment before two years of age was not significantly (borderline) associated with current asthma (aOR 2.00 (0.98 – 4.12), p=0.058) (paper IV).

Propensity modelling

To assess current asthma at 10 years of age for rBO children, the propensity modelling was used to adjust for severity of disease at two years of age (see section on statistical methods). The propensity scores were based upon gender, parental atopy and severity score at two years of age. The analysis resulted in a non-significant aOR (95% CI) 1.842 (0.889-3.815), (p=0.10), for the use of ICS compared to no use before two years of age. In this model, the propensity score at two years of age was significantly associated with current asthma at ten years of age (aOR 7.51 (1.70 – 33.21), (p=0.008) (paper IV).

13 GENERAL DISCUSSION

13.1 Prevalence of childhood asthma, wheeze and allergic sensitization in children

Prevalence of recurrent bronchial obstruction in 0-2 year old children

The prevalence of rBO until two years of age was approximately 8.3% of children in the present birth cohort study (paper III). The knowledge about prevalence of asthma and asthma-related symptoms in children younger than two years of age is limited. A recently conducted cross-sectional survey (88) by telephone interviews to parental caretakers of child population aged 1-5 years in the USA and Europe found that 32% children have been reported to suffer from cough, wheeze or breathlessness in the preceding six months. However, the survey was conducted during the winter months, the period when children often have infection-triggered wheezing. In this report (88), prevalence varied from 29% in Northern Europe to 48% in Southern Europe whereas it was 27% in the USA. Furthermore, 24% of the interview population (i.e., 8% of the overall population) suffered persistent symptoms during the previous six months despite current treatment. The apparent discrepancies in the prevalence rates in the study by Bisgaard et al (88) and the present study (paper III) could be due to differences in study design, longitudinal versus cross-sectional, age range of the children and the time period when they were studied. Our study focused on the children from 0 to 2 years of age instead of 1-5 years in the study by Bisgaard et al (88). Furthermore, the children in our study had *recurrent* symptoms of bronchial obstruction in a birth cohort population in contrast to the study by Bisgaard et al (88). The results from prospective cohort studies are comparatively more reliable and valid when compared to the results from cross-sectional surveys. However, more prospective studies are needed to verify these findings.

Prevalence of asthma in school children

In 10-year-old children in the ECA birth cohort study in Oslo, the prevalence of asthma ever

was 20.2 %, doctor's diagnosis of asthma 16.1%, current asthma 11.1 % and wheeze ever 30.3% reported in 2005. Asthma prevalence has continued to increase among children in Norway since the first report of 0.4% in 1948 (127). Published reports from cross-sectional studies in Norway demonstrated lifetime asthma prevalence of 1.77% in 1954 (128), 3.1% in 1981 (129) to 8% in 1993 (26) and 9.3% in 1994 (28). The three last studies used identical questionnaires within Oslo, in contrast to earlier studies which employed different questionnaires. The present study indicates more than doubling of prevalence rates over the last 10 years until the present finding of 20.2%. This is supported by a repeated cross-sectional study in Danish school children which has reported doubling of asthma prevalence from 1983 to 2001 (130). In a report from ISAAC Phase-III conducted worldwide, Asher and co-workers (7) found increasing prevalence of asthma in 6-7 year old children compared to 13-14 year age-group during the period 2002-3. Furthermore, Pearce and colleagues (8) using ISAAC questionnaires found increases in asthma prevalence in Africa, Latin America, and parts of Asia indicating that the global burden of asthma is continuing to rise.

Prevalence of wheeze

The high prevalence of wheeze ever (30.3 %) reported in 10-year-old Oslo children (paper I) is comparable to that of prevalence rates reported in the British studies from the 1990s. Furthermore, a cross-sectional study in Norway by Tollefsen and colleagues (21) have shown corresponding prevalence rates of current wheeze, 29.0% and 33.5% among girls in age groups 13-16 and 17-19 years respectively, and 20.4% and 22.1% among boys in the same age group.

Prevalence of allergic skin sensitization

The finding of SPT to minimum one allergen in 29.3% of 10 year old children has corresponded to one previous Swedish report by Hesselmar and colleagues (131). They found positive SPT in 31.7% of 12 -13 year old Swedish children in 1996 with regional

variations, 22.5% in Gothenburg to 38.1% in Kiruna (131). However, the prevalence of asthma was found to be 8.5% in their study. Hence, our data compared to the Swedish study (131) suggest that the increase in prevalence of childhood asthma in Oslo has not been accompanied by a parallel increase in allergic sensitization. This is also supported by findings in a Danish study conducted 15 years apart (35).

13.2 Use of inhaled corticosteroids in early childhood

The extent of use of ICS in young children with asthma or asthma-like symptoms is not well known. To our knowledge, the study reported in paper II was the first report of extent of ICS use in a prospective follow-up cohort study in very young children. During the study period 1992-93, 2.1% of all children had received ICS before two years of age, and 21% of all children with rBO had at some time by two years of age been treated with ICS. The reported use of ICS in infants and young children with rBO in the community of Oslo was likely to be representative of the use in the Scandinavian countries at that time.

An epidemiological study in Japan (132) assessed the use of ICS between 1990 and 2002 and information was extracted from the prescription database in four age groups (0-4,5-19,20-39 and 40-64 years). Authors found that the number of prescriptions for ICS per year increased in all age groups except those aged 0-4 years. This might suggest that there might still be concerns regarding the efficacy and safety of ICS in this age group, even though reassuring studies are available (133). In a Swedish study Wennergren et al (134), there was an observed decrease in acute asthma hospitalizations in children > 4 years which was probably due to ICS use (based upon prescription statistics). In their study, the hospital admissions for acute asthma in those < 4 years, however, had not decreased, but the prescription rates for ICS use in this age group was not stated.

13.3 Effect of inhaled corticosteroids on lung function in early childhood

The data presented in paper II, to the authors' knowledge, is the first study to assess the possible modifying effect of ICS on lung function in infants and very young children with rBO in a non-interventional, observational prospective cohort. As reported in paper II, we found that steroid naïve children with rBO who later received ICS treatment had reduced lung function on the first visit (around 11 months of age) compared to those who did not, but the differences in lung function disappeared by two years. The positive change in baseline t_{PTEF}/t_E between the first and second visit among the ICS treated children was positively correlated with the duration of inhaled steroid treatment.

A systematic review by Calpin et al (135) which included 10 randomised double-blind, placebo-controlled studies in preschool children concluded that ICS were effective in controlling childhood asthma. In infants and young children with recurrent BO after admission to hospital due to acute bronchiolitis, several studies demonstrate the beneficial effects of ICS (136-138), whereas others did not (139). Teper and colleagues (100) in a double-blind RCT of children with recurrent wheezing younger than two years of age found that treatment with fluticasone 125 micrograms twice daily for six months improved lung function (*V'*max FRC) and clinical outcomes in children with wheezing compared to group treated with placebo. Most of these studies showed effects during the treatment phase. In contrast, some recent studies (95;101) have shown lack of effect during the treatment-free period. Guilbert and colleagues (95) found improved measures of lung function (derived from impulse oscillometry) in preschool children at high risk for asthma during the fluticasone treatment phase of two years but the effect had disappeared during the treatment-free third observation year (95). Murray and co-workers (101) could not find improvement in lung function (specific airways resistance, forced expiratory volume in one second and airway reactivity) after treatment with fluticasone in preschool children who were

prospectively followed for five years.

Diminished lung function and severity of illness

In the study reported in paper II, lung function was reduced in the rBO children who later received ICS compared to those who remained steroid naïve, and also compared to controls at the time of enrolment (mean age 11 months) into the case-control study. To our knowledge, this has not been previously reported in children younger than one year of age. This finding indicates that children with lower lung function are probably more disposed to severe recurrent bronchial obstruction and thereby necessitates the treatment with potent anti-inflammatory agents like ICS. The findings in paper II further show that inhaled steroids in general were given to children with the lowest lung function, albeit the prescribing physicians were ignorant of the lung function results. This observation strengthens the likelihood that these lung function measures reflect severity of OAD in young children, as previously reported (65;108).

There is potential risk of regression towards mean if extreme values of lung function were selected (126) in the study reported in paper II. The analyses demonstrated that this was not the case (126). Regression towards mean was also unlikely in view of the marked differences between cases and controls in lung function change from the first to the second visit. However, it should be noted that the groups of children selected were not on the basis of lung function values, but on the treatment received.

13.4 Severity of obstructive airways disease by two years and later asthma

Early identification of children at risk of persistent asthma in childhood may improve follow-up of the children at risk. To explore further how severity of OAD in early life has impact on later occurrence of asthma, we have developed a simple severity scoring system for children with recurrent bronchial obstruction at two years of age based upon commonly available clinical data and no laboratory tests.

In the results reported in paper III, a asthma severity score calculated by frequency, persistence of BO and hospitalisations for BO during the first two years of life predicted a higher risk for current asthma at 10 years of age. The high risk for current asthma at 10 years in children with higher severity scores (above five) compared to children with no BO by two years of age, exceeds previously published reports (58;59;140). A severity score of five is not infrequent in clinical practice, reflecting two hospital admissions with one additional obstructive episode or alternatively 5-6 episodes of BO with one month of persisting symptoms.

There were two studies (58;59) in the literature which attempted to predict later wheezing/asthma in the children, the first study only after 12 months follow-up (59) whereas the second study developed predictive indices to predict asthma beyond one year (58). Clough and co-workers (59) in a study of 107 preschool children found that after 12 months follow-up of 3-36 months old children, that wheeze was more likely to persist in older, atopic infants with parental atopy, and they also detected asthma requiring prophylactic anti-asthmatic treatment in 49.5 % of the children. In their study (59), among different predictive models, the model offering best prediction of persistent wheeze with least risk of including asymptomatic subjects was age at presentation and soluble interleukin-2R.

Severity score versus asthma indices

The Tucson Children's Respiratory Study (58), as one of the few studies with reported clinical indices based upon the assessment of severity of illness to predict asthma beyond 1-2 years, employed factors from 0-3 years of age to predict asthma in school age. The loose and stringent asthma indices developed by Castro-Rodriguez et al (58) were applied to data obtained before three years of age to detect active asthma during one of the surveys between six and 13 years of age. The ORs for active asthma at 11 years were 2.6 and 4.3 for the loose and the stringent indices respectively (58). In the study reported in paper III, the ORs of

current asthma for children with severity score above five compared to children with no BO was higher (7.9) than when we applied the loose and stringent (6.4) indices suggested by Castro-Rodriguez et al (58).

Severity score and prognosis

The predictability of severity score at two years classified by current asthma at ten years was demonstrated by a statistically significant area under the curve by the ROC analysis. ROC analyses as well as assessment of positive and negative predictive values demonstrate that the scoring system can be used to identify children with high and low risk of active asthma in later childhood. Consequently, the information obtained in early life may have a prognostic value for the patient. Although high severity score can predict later disease, it can not be used as a prognostic score as a part of asthma management strategy in order to prevent later illness. However, scores to suggest prognosis may be useful to identify children with special needs for closer follow-up and the management of disease.

The simple scoring system based solely upon the history of OAD by two years in paper III, may be as suitable to predict current asthma eight years later as the predictive indices (58;59) which includes heredity and invasive laboratory tests in this age group. However, compared to the severity score at two years of age, a severity score calculated at 12 months of age, although a significant risk factor had a poor predictive capability for current asthma nine years later. The low predictability of the 12 month severity score in the study reported in paper III may reflect that many young children had not yet developed manifestations of OAD. However, it should be pointed out that the score at 12 months of age was constructed from data obtained in questionnaires only, with less detailed information than that obtained during parental interviews at two years of age.

Severity score and BHR

The results reported in paper III demonstrate that children with higher severity scores above

five by two years of age had significantly more often severe BHR compared to children with severity score of 0. To the authors' knowledge, association between severity of OAD in early life and later BHR in children has not been reported previously. To improve our understanding of the mechanisms involved between severity of OAD in early life and later development of BHR and asthma, more prospective studies are warranted.

13.5 Early inhaled corticosteroids treatment and prognosis on later asthma

The main purpose of the study reported in paper IV was to assess if early ICS treatment has any effect on disease progression with relation to occurrence of asthma by 10 years of age. We found no evidence that early use of ICS in children with rBO during the first two years of life reduced the occurrence of current asthma at 10 years of age. To our knowledge, this is the first reported risk assessment of early ICS treatment for current asthma eight years later in an observational birth cohort study.

The finding in paper IV that ICS treatment before age two years did not reduce ongoing asthma at 10 years of age was supported by three recent RCTs (95;101;141) with ICS treatment in early childhood with a follow-up to three and five years of age. Guilbert et al (95) studying high risk children (positive asthma predictive index) reported significant improvement in lung function measures during two years treatment with inhaled fluticasone propionate 88 µg twice daily compared to placebo. However, during the third treatment-free year, there was no longer any improvement either in asthma symptoms or exacerbations or lung function (95). Murray and colleagues (101) in a birth cohort treated 200 children with fluticasone 100 µg twice daily or placebo and attempted three monthly treatment reductions before eventual cessation of ICS treatment. After a follow-up until five years of age, the authors found no effect of fluticasone on the natural history of asthma, lung function decline or airways reactivity after stopping treatment (101). In the study of Bisgaard et al (141) of high risk infants from one month of age, daily treatment of 400 µg budesonide for two weeks

intermittently during wheezy episodes or cough was no better than placebo to prevent persistent wheeze at three years of age. Two of these studies introduced treatment early (101;141), whereas Guilbert et al (95) started treatment after two years of age. The treatment strategies also differed in the three studies: two years continuous ICS treatment in the study by Guilbert et al (95), a step-up/step-down strategy in the study by Murray et al (101) and short courses of ICS in the study by Bisgaard et al (141). In contrast to these studies (95;101;141), the ICS treatment in the present observational and non-interventional study was decided by their regular physicians based on clinical symptoms (paper IV).

The protocol for all these trials (95;101;141) and the study reported in paper IV differs in several aspects. However, the negative results provide additional, non-overlapping evidence that ICS do not change the natural course of asthma. The fact that the long term results of all these studies with respect to asthma prevention were identical in spite of their disparate target populations and treatment strategies allows for reasonably definitive conclusions. However, apart from the study of Guilbert et al (95), Murray et al (101) and the present study (paper IV), little information of long-term effects of ICS treatment during the first few years of life is reported.

The concept of inflammatory remodelling in early childhood is still unclear as reticular basement membrane thickening and the eosinophilic inflammation characteristic of asthma in older children and adults have not been found in symptomatic children below three years of age with reversible airflow obstruction (80). Moreover, evidence of persistent inflammation despite inhaled corticosteroid therapy has been reported in adults (142) and adolescents despite asthma in remission (143).

Factors in early life associated with more severe disease are both risk factors for persisting disease (paper III), as well as for starting ICS treatment. Although subjects who received ICS treatment by two years had more severe disease, the propensity score was used to adjust for

the severity of the disease between 0-2 years of age. The propensity approach underlined the result found with a conventional analysis.

13.6 Strengths and limitations of the present studies

Strengths

The strength of the present birth cohort is the detailed and well described population that has been followed for 10 years with a design especially suited for investigating asthma and asthma phenotypes. Furthermore, the present cohort includes a large population of children with lung function measurements shortly after birth.

The results reported in papers III and IV are from children enrolled in nested case-control design at two years of age, a part of an observational prospective cohort of healthy newborn children. This study with a follow-up rate of 89 % at 10 years of age is based on an urban, unselected birth cohort, with early identification of OAD as well as children with no lower respiratory disease before two years of age. The children were prospectively followed from birth to two years with six monthly questionnaires to increase the reliability of data and thereby limiting the risk of recall bias for the first two years. The study has focused on clinical presentation at the time of investigation (two and 10 years), rather than the retrospective categorization of "wheezing" phenotypes such as early onset transient wheeze, persistent wheeze and late onset wheeze (3;40), which in our view is more appropriate to do in such study.

Clinical examinations of all children were performed by study doctors with experience in paediatric pulmonology and allergology. The clinical assessments can therefore be regarded as reliable and consistently reported.

In papers I, III and IV, current asthma was defined by combining clinical diagnosis, symptoms and medication and a physiological objective measure of BHR increasing the

likelihood of selecting children with true persistent asthma and ongoing inflammatory airways disease. Furthermore, we chose to define asthma more strictly than a doctor's diagnosis alone, or by questionnaire-reported wheeze. This is in contrast to other published studies where symptoms and/or treatment alone were used to define current asthma. Thus, a detailed structured interview and the requirement of at least two out of three commonly used criteria reduce the risk for both over- and under- diagnosis of asthma and improve the quality of asthma diagnosis.

The design of the study ensured that treatment was decided by the child's own doctor, independent of the study doctors, thus avoiding the risk of observational bias in the treatment assignment (paper II and IV).

Limitations

In the observational data sets presented in the paper IV, we could not separate the influence of the start of treatment from the effect of the duration of treatment. Subjects with severe disease are more likely to receive treatment and more likely to have a worse outcome. Propensity score methodology is useful in an observational study like the present (paper IV) and in situations where a RCT is not feasible (144). The purpose of the propensity score is to adjust for disease severity, and it is therefore important, to explore a number of possible propensity models and achieve a model that accurately predicts treatment assignment in order to avoid selection bias (125). The propensity modelling has been recommended by the United States Food and Drug Administration (FDA) for use in non-randomised trials, to compensate for the problem that patients are not randomly assigned to treatment groups with equal probability (124). The results from these two types of studies (RCT and cohort study) complement each other. On one hand, the results from a non-randomised observational study may generate a hypothesis and may provide useful supportive data but long term RCTs will be needed to confirm such findings. On the other hand, prospective cohort studies can also be

useful to confirm the findings from RCTs.

The results from RCTs has highest level of evidence (Evidence category A) when compared to observational studies (Evidence C) as reported by GINA (3). Although RCTs are generally considered the best study designs to assess treatment efficacy, they however are associated with high costs, selected populations with stringent inclusion and exclusion criteria tailored to the medicament studied and are often logistically difficult to maintain for many years. In contrast to RCTs, the advantages of observational cohort studies are that they reflect 'real life' clinical practice and includes broader population of participants in the absence of disease-related exclusion criteria (145). For medical practice, the results from longitudinal observational studies are reliable especially concerning diagnosis and prognosis (146).

14 CONCLUSIONS

1) The prevalence of recurrent bronchial obstruction in young children by two years of age was 8.3 % in the present birth cohort.

Furthermore, every fifth 10 year old child in the city of Oslo at some time had asthma. Our finding of 20.2 % lifetime prevalence of asthma among 10-year-old children represents the highest number ever reported in Scandinavia. However, the observed increase in asthma prevalence in this cohort study was not accompanied by parallel increase in allergic sensitization.

2) The extent of use of ICS (reported by parents) corresponded to 2.1 % of all children in the cohort study followed until two years of age and 21 % of children with recurrent bronchial obstruction by two years of age.

3) The rBO children who received subsequent ICS treatment had reduced lung function compared to those who remained steroid naïve at debut of obstructive airways disease. Lung function improved in children using ICS until two years of age, mostly in children with the longest duration of treatment. It suggests that ICS treatment once started has a disease modifying effect with relation to lung function in young children. This factor should, therefore, also be taken into consideration when addressing the effects of environmental factors upon lung function.

4) A scoring system based on severity and frequency of obstructive airways disease during the first two years of life predicted a higher risk for current asthma at 10 years of age. The simplicity of the symptom score renders it generally applicable for early identification and follow-up of children at high risk for persistent asthma. Furthermore, the children with high severity score (above five) by two years of age had significantly more often severe BHR (PD_{20}-metacholine < 1 μmol) at 10 years of age than children with a lower or 0 score.

5) A mean of nine months treatment with ICS in children with obstructive airways disease

before the age of two years does not seem to reduce the occurrence of asthma at 10 years of age, but larger intervention studies are needed to confirm this finding. Although early use of ICS has been shown to have short term beneficial effects, the results presented in paper IV study does not support the hypothesis of improved long term prognosis for children treated with inhaled corticosteroids for recurrent bronchial obstruction in early life. Thus, the possible role of ICS treatment as a disease modifier in the development of asthma in children is yet to be ascertained.

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ERRATA

The following changes have been made from the thesis originally submitted to the Doctoral Committee:

1. List of Papers, page 7: updated reference of Paper III. Since submission to the Doctoral Committee, paper III has been published in Thorax which is now included as a part of thesis. There are no changes between the online version that was originally submitted and the final published version.
2. Replaced “six” with “five” on page 44, line 5.

PAPERS I - IV